THE CHEMISTRY OF FLESH—METHODS FOR THE DETERMINATION OF CREATININ AND CREATIN IN MEATS AND THEIR PRODUCTS.

BY

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(FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.)

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Dr. Myron Hardman

THE CHEMISTRY OF FLESH.

(Fifth paper.)[1]

METHODS FOR THE DETERMINATION OF CREATININ AND CREATIN IN MEATS AND THEIR PRODUCTS.

BY H. S. GRINDLEY AND H. S. WOODS.

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In the previous papers from this laboratory it has been conclusively proven by improved methods of analysis that meats contain a considerably greater quantity of so-called organic extractives than previous investigations had shown. It has also been demonstrated that a considerable proportion of flesh is soluble in cold water; that the cold water extraction of meat can be made complete and that it is possible to determine with accuracy the total nitrogenous organic extractives of such extracts. In connection with the researches of this laboratory upon the chemistry of flesh it became desirable to devise means to ascertain the relative proportion of the different components which go to make up these extractives. The creatin and also the creatinin if present in meats would undoubtedly be completely extracted by cold water. That being true, an attempt was made to apply the colorimetric method of Folin[2] to the estimation of the creatinin and creatin, using the Duboscq colorimeter.

Tests for Creatinin in Meats.

In the first place the method was used to test for creatinin in a cold water extract of chicken flesh. Two hundred cubic centimeter portions of the extract representing 4.112 grams of fresh

[1] *Journ. Amer. Chem. Soc.*, xxvi, p. 1086, 1904; xxvii, p. 658, 1905; xxviii, pp. 25 and 469, 1906.

[2] *Zeitschr. f. physiol. Chem.*, xli, p. 223, 1904; *Amer. Journ. of Physiol.* xiii, p. 48, 1905.

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chicken flesh were tested directly for creatinin in the manner described by Folin in estimating this constituent in urines. The tests gave only the slightest indications, if any, of the presence of creatinin. When the alkaline picrate solution of the extract of the chicken flesh was allowed to stand for a few days, it turned red, the color growing more intense each day. Water extracts of beef and solutions of meat extracts when treated directly with the alkaline picrate solution behaved in a similar manner, showing that creatin under these conditions is gradually converted into creatinin even in alkaline solutions. This is contrary to the supposed behavior of these two nitrogenous bodies in the presence of alkaline solutions. The true nature of this reaction is now being further studied in this laboratory.

In order to prove further the presence or absence of creatinin in aqueous extracts of fresh flesh, 400 c.c. of a cold water extract of beef, representing all of the soluble constituents from 8 grams of lean beef round, were tested directly for creatinin as above by Folin's method. The tests gave no trace of creatinin. The alkaline picrate solutions of these beef extracts upon standing for a few hours gradually turned red, as did the extracts from chicken flesh. These experiments, which have often been repeated, demonstrate that the work and conclusions of Toppelius and Pommerehne,[1] and later that of Emil Wörner,[2] were correct, and that the investigations of G. S. Johnson,[3] which led him to the deductions that perfectly fresh meats contain creatinin, but no creatin, were wrong. As judged by Jaffe's reaction fresh meats contain only the slightest trace of creatinin if any at all. However, the creatin of flesh is in part at least very readily changed to creatinin. The following experiment demonstrates this fact. Three 500 c.c. portions of a cold water extract, each representing 10.015 grams of chicken flesh, were carefully evaporated upon a water-bath to a volume of 50 c.c. in order to completely coagulate the soluble proteid matter. The solutions were filtered, the precipitates thoroughly washed, and the filtrates evaporated to a volume of 75 c.c. They were then tested for

[1] *Arch. de pharm.*, ccxxxiv, p. 380, 1896.
[2] *Zeitschr. f. physiol. Chem.*, xxvii, p. 1, 1899.
[3] *Proc. of the Roy. Soc.*, l, p. 287, 1901.

creatinin and they gave readings which represented 0.08, 0.11, and 0.15 per cent. of creatin, calculated upon the basis of the fresh substance of the flesh. This experiment and a number of others which we have made prove that the evaporation of an aqueous extract of flesh upon the water-bath, in the presence of its natural acidity, changes creatin to creatinin. It is just possible that this action may be one influence which produces flavor during the cooking of meats. This subject is now being studied in connection with these researches.

Estimation of Creatin in Meats.

The estimations of creatin in the aqueous extracts of flesh were made by taking 500 c.c. portions of the solution, evaporating and filtering as mentioned above to remove the coagulable proteids. The solutions thus obtained were treated with hydrochloric acid and evaporated upon the water-bath to a small volume. This treatment was repeated with the addition of water until all the creatin had been changed to creatinin. The solutions were then made up to exactly 100 c.c. and 30, 40, and 50 c.c. of the same were used to make the quantitative test with the colorimeter.

Table No. I gives a few determinations of creatin in different kinds of flesh.

TABLE I. CREATIN IN FRESH FLESH.

No. of Sample.	Kind of Meat.	Weight of Sample.	Reading of Colorimeter.	Weight of Creatinin due to Creatin. (A)	Weight of Creatin (A × 1.16)	Creatin.
		Gr.	Mm.	Mgr.	Mgr.	Per cent.
2075	Beef Round	2.8066	8.2	9.88	11.46	0.41
2084	Cold Storage Fish	3.8369	7.9	10.25	11.89	0.31
2085	Cold Storage Fish	4.6625	7.8	10.38	12.04	0.26
2110	Refrigerated Chicken	4.0084	8.1	10.00	11.60	0.29
2111	Refrigerated Chicken	4.9152	8.4	9.64	11.18	0.23
2112	Refrigerated Chicken	5.0262	7.8	10.38	12.04	0.24

As a result of the preliminary experiments here reported, the following method is recommended for the estimation of creatin in flesh. Prepare the aqueous extract of the flesh as described in a former paper[1] from this laboratory.

Evaporate 500 c.c. portions of the extracts to 50 c.c., filter, wash the coagulated proteid thoroughly with hot water, add 25 c.c. of $\frac{N}{10}$ hydrochloric acid to each of the filtrates, and evaporate them to a volume of 10 to 15 c.c. Now add 50 c.c. of water and 10 c.c. of $\frac{N}{10}$ hydrochloric acid and again evaporate to a volume of 10 to 15 c.c. Care must be taken to insure the complete change of creatin to creatinin. Cool the solutions, transfer them to a 100 c.c. measuring flask, and dilute to the mark. Transfer an aliquot portion of this latter solution, equivalent to 200 c.c. of the original extract, to a 500 c.c. flask, add 15 c.c. of a 1.2 per cent. (saturated) picric acid solution and 5 c.c. of a 10 per cent. sodium hydroxide solution, shake well and allow the solution to stand for five minutes. In the meantime practice reading the colorimeter, using an $\frac{N}{2}$ solution of potassium bichromate in each cylinder. Now dilute the alkaline creatinin picrate solution to 500 c.c., mix thoroughly, rinse one of the cylinders well with this unknown solution, add a portion of the solution to the cylinder, and make several careful readings at once, comparing the unknown solution with 8.0 mm. of the bichromate solution. According to Folin, the correct reading in millimeters divided into 81 gives the number of milligrams of creatinin present. This figure multiplied by 1.16 gives the number of milligrams of creatin. If an aliquot portion equivalent to 200 c.c. of the extract does not give a satisfactory reading, use such a portion as will give a reading of about 8 mm.

This method has been used successfully for the determination of creatinin and creatin in uncooked and cooked meats and in meat broths and meat drippings.

Creatinin and Creatin of Commercial Beef Extracts.[2]

The nature of a considerable proportion of the constituents of

[1] *Journ. Amer. Chem. Soc.* xxvii. p. 658, 1905.

[2] After the data herein recorded had been obtained, and this paper had been written, the October number of the *Journal of the American Chemical Society* was received, containing the paper of Bigelow and Cook,

commercial beef extracts is still unknown. A ready method for the estimation of creatinin and creatin in these products would undoubtedly be useful in determining the commercial value and the grade of the various commercial beef extracts and similar preparations which are now upon the market. As Folin's method for the estimation of these substances in urines proved to be an excellent means for the determination of these nitrogenous constituents which are contained in aqueous extracts of flesh, we undertook to apply it to the determination of these bodies in commercial extracts. The method has proved equally successful in this connection. Its application here has demonstrated that commercial beef extracts contain both creatinin and creatin. The details of the procedure in the case of the extracts are entirely similar to those outlined above for cold water extracts of flesh. The results of a number of determinations of creatinin and creatin in a number of the prominent commercial extracts now on the market are given in the following tables.

TABLE II. CREATININ IN COMMERCIAL BEEF EXTRACTS

No. of Sample.	Weight of Sample.	Reading of Colorimeter.	CREATININ.	
			Weight.	Per cent.
	Gr.	Mm.	Mgr.	
1	0.3008	7.8	10.38	3.45
2	0.2545	8.6	9.42	3.70
3	0.5800	8.1	10.00	1.72
4	0.7221	8.0	10.13	1.40
5	0.1972	7.8	10.38	5.27
6	0.7234	8.2	9.88	1.37
7	0.8206	8.2	9.88	1.20
8	0.5458	7.7	10.52	1.93
9	1.1391	8.6	9.42	0.83
10	0.4720	8.1	10.00	2.12
11	0.6786	8.5	9.47	1.39
12	0.7540	7.5	10.08	1.30
13	0.2774	7.3	11.10	4.00

upon "The Separation of Proteoses and Peptones from the Simpler Amino Bodies." In this paper, the above authors mention the fact that they have applied the method of Folin for the determination of creatin in meat extracts, but they give no analytical results. Notwithstanding this preliminary notice of Bigelow and Cook, it was deemed advisable

TABLE III. CREATIN IN COMMERCIAL BEEF EXTRACTS.

No. of Sample	Weight of Sample.	Reading of Colorimeter.	Weight of Original Creatinin plus Creatinin due to Creatin.	Weight of Original Creatinin (See Table I.)	Weight of Creatinin due to Creatin (A)	Weight of Creatin A × 1.16	Creatin.
	Gr.	Mm.	Mgr.	Mgr.	Mgr.	Mgr.	Per cent.
2	0.1833	7.9	10.25	6.78	3.47	4.03	2.18
3	0.2636	7.7	10.52	4.55	5.97	6.93	2.63
4	0.1850	7.9	10.25	2.60	7.65	8.74	4.79
5	0.1512	8.4	9.64	7.96	1.68	1.95	1.29
6	0.1841	8.6	9.42	2.38	7.04	8.17	4.35
7	0.1641	8.0	10.13	1.98	8.15	9.45	1.20
8	0.2593	8.0	10.13	5.00	5.13	5.95	2.29
9	0.7088	8.8	9.20	5.86	3.34	3.87	0.55
11	0.3016	5.0	16.20	4.19	12.01	13.93	4 62
12	0.2262	6.85	11.82	2.94	8.88	10.30	4.55
13	0.2080	7.00	11.57	8.32	3.25	3.77	1.81

TABLE IV. COMBINED CREATININ AND CREATIN IN COMMERCIAL BEEF EXTRACTS.

No. of Sample.	Creatinin.	Creatin.	Sum of Creatinin and Creatin.
	Per cent.	Per cent.	Per cent.
2	3.70	2.18	5.88
3	1.72	2.63	4.35
4	1.40	4.79	6.19
5	5.27	1.29	6.56
6	1.37	4.35	5.72
7	1.20	1.20	2.40
8	1.93	2.29	4.22
9	0.83	0.55	1.38
11	1.39	4.62	6.01
12	1.30	4.55	5.85
13	4.00	1.81	5.81

These results show that there are marked differences in commercial meat extracts as to their content of creatinin and creatin. In some of the extracts, creatinin occurs in considerably

to publish our paper in full and without any modifications, since it gives data which prove without doubt that the method of Folin may be applied successfully to the estimation of creatinin and creatin in meats, in meat broths, in meat extracts, and in other meat products. We have been using this method continuously in our investigations upon the chemistry of flesh since March 15, 1906.

greater quantities than does creatin, while in others the amounts of the latter constituent are greater than the amounts of the former. It is difficult to state at present the cause or causes which, in the preparation or keeping of the commercial extracts, produce this variation in the proportion of these two nitrogenous constituents. Experiments upon meat extracts prepared upon a small scale in the laboratory, from fresh meat, are now under way to find out if possible the conditions which produce the above difference in the relative proportion of creatinin and creatin.

The data here given also show that there is a marked difference in the total amount of creatinin and creatin contained in different commercial extracts now upon the market. Further study will probably show that this difference in the combined amount of these nitrogenous constituents is due, in the main, to the material from which they are manufactured. We hope to be able to study this phase of the work in the near future.

We wish here to express our thanks to Mr. H. H. Mitchell for his valuable assistance in connection with the analytical work involved in this paper.

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